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Identification of *Triticum durum* genotypes showing increased tolerance to oxidative stress

Identyfikacja genotypów *Triticum durum* o zwiększonej tolerancyjności na stres oksydacyjny

Summary. Oxidative stress can significantly impair the plants growth and development. It can be triggered by various biotic and abiotic factors. The study analyzed durum wheat genotypes aiming at identifying the forms characterized by increased tolerance to stress induced by methyl viologen (paraquat). The presence of paraquat in the medium caused the majority of analyzed forms to reduce the weight and length of the shoot part of seedling. In addition, inhibition of the root system development compared to control forms was observed. In some of the forms studied, oxidative stress caused chlorosis. Six different types of responses to oxidative stress were found. Most genotypes (58.8%) showed a reduction in seedling weight and length, irrespective of the paraquat concentration used. Nine genotypes resistant to stressor (CYP, MEX × 2, ETH, FRA, ITA, POL, SUN, TUN) were identified, which constituted 6.1% of the examined forms.

Key words: durum wheat, oxidative stress, methyl viologen, paraquat

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is an important cereal, the grains of which are mainly used for the production of pasta, couscous and bulgur groats [Kubaláková et al. 2005]. Durum wheat grain is characterized by a high degree of vitreosity and hardness, high content of total protein and high content of gluten and yellow pigments.

The disadvantage of durum wheat is its susceptibility to biotic and abiotic stress, which in turn can lead to oxidative stress [Rachoń et al. 2011, Brankovic et al. 2014].

Oxidative stress is defined as the imbalance between the production of reactive oxygen species (ROS) and their removal in a given organism or cell [Sheu et al. 2006]. Many different factors contribute to the formation of oxidative stress, which, if severe or prolonged, can cause a plant death. It can be caused, for example, by prolonged drought, frost, excessively high temperature, high salinity, attack by pathogens, injury, use of herbicides or pesticides and the presence of heavy metals [Mittler 2002, Ahmad et al. 2008].

The increased amount of ROS leads to changes in the structure of DNA, such as the double-strand breaks or modification of nucleobases [Małecka and Tomaszewska 2005]. Reactive oxygen species during protein interactions can cause polypeptide chain rupture, modification of amino acid residues, modification of prosthetic groups, change in the charge, and change in the structure of proteins. ROS mainly act on amino acids containing a sulfur group (cysteine, methionine). Consequently, these proteins may lose their biological functions and may undergo faster proteolysis [Puzanowska-Tarasiewicz et al. 2008]. Reactive oxygen species also cause the lipid peroxidation in membranes. Free radicals initiate a chain response of lipid oxidation, which may cause modification of physical properties of cell membranes, e.g. lowering membrane potential or increasing membrane permeability [Nowicka and Kruk 2013].

As a defense response, plants created a system consisting of proteins having the nature of antioxidant enzymes and non-enzymatic components that participate in the elimination of reactive oxygen species. The main antioxidative enzymes include superoxide dismutases, catalases and peroxidases, while non-enzymatic components include, but are not limited to, glutathione (GSH), cysteine, ascorbate, α-tocopherol [Małecka and Tomaszewska 2005, Jia et al. 2013, Westernack and Hause 2013].

The subject of this study was to assess the resistance of durum wheat collection to oxidative stress and to identify genotypes characterized by increased tolerance to generation of reactive oxygen species. As a factor inducing the oxidative stress, paraquat, commonly referred to as methyl viologen (1,1'-dimethyl-4,4'-bipyridinium dichloride) was used. This organic compound is applied in plant research – it catalyzes the formation of superoxide radicals [Docherty and Kulpa 2005].

MATERIAL AND METHODS

The research material comprised 148 cultivars and breeding lines of durum wheat (*Triticum durum* Desf.) originating from the collection of the Institute of Plant Genetics, Breeding and Biotechnology of the University of Life Sciences in Lublin (Appendix A).

Before starting the experiment, the kernels were sterilized with chlorine gas. Forty kernels from each durum wheat form were placed in 15 ml falcons. The unscrewed falcons were placed in a tripod and placed in a plastic container. 100 ml of ACE disinfectant and 3 ml of 38% hydrochloric acid were poured into the glass cuvettes placed

in the container. The container was immediately closed with a plastic lid. Sterilization with evolving chlorine lasted 4 h.

Tests to assess the resistance of a given form of durum wheat to oxidative stress were performed in several cycles. To induce germination, the sterilized kernels were placed in Petri dishes with solid sugar-free MS medium and placed in a refrigerator at 4°C for 3 days. Then the dishes containing sprouted kernels were placed in a phytotron (temperature 24°C, photoperiod 16/8 h). Three-day-old seedlings were transferred to jars with sugar-free solidified MS medium containing 5 or 10 μM paraquat (methyl viologen – MV). The control consisted of plants growing on medium without paraquat. Jars with plants were placed in a phytotron. After six days of oxidative stress, the plants were measured and weighed.

Data obtained during phenotypic screening was subjected to statistical analysis using the Statistica 13.1 software. To determine the significance of differences between group means in the variance analysis system, Tukey's HSD post-hoc test was used at the significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The presence of paraquat in the medium caused the majority of analyzed forms to reduce the weight and length of the shoot part of seedling, as well as inhibiting the development of the root system compared to control forms. In addition, in some of the forms studied, oxidative stress caused chlorosis. Plant response to paraquat stress in the form of chlorosis has also been observed in studies on other species [Ross et al. 1979, Youssefian et al. 2001, Cui et al. 2019].

Differences in the response of durum wheat seedlings to oxidative stress caused by the presence of methyl viologen were observed. The response of some forms was dependent on the concentration of the stress factor (either 5 or 10 μM paraquat). Some forms reacted the same regardless of the paraquat concentration applied. As a result of the analysis, the obtained data identified 6 types of response of durum wheat seedlings to a given oxidative stress (Tab. 1). Some forms were characterized by a specific pattern of responses that did not allow them to be assigned to any of the defined types of seedling responses to oxidative stress. Statistical differences between group means of tested material are shown in Appendix B.

The first type of response included genotypes resistant to methyl viologen (Fig. 1). These forms were characterized by the same growth under conditions of induced stress as under control conditions. No effect of paraquat on both weight and length of seedlings was observed, irrespective of the MV concentration used. Forms characterized by resistance to the oxidative stress applied constituted 6.1% of the tested genotypes. The resistant material was of diverse origin (CYP, MEX × 2, ETH, FRA, ITA, POL, SUN, TUN).

Table 1. Types of response of durum wheat seedlings to oxidative stress caused by the presence of paraquat

Type No.	Response type	Designation of the form in the collection
I	No response to oxidative stress, regardless of the MV concentration used	10, 30, 42, 47, 50, 160, 1108, 1114, 1125
II	Reduction of weight and length of seedlings treated with higher MV concentration	1057, 1101
III	Seedlings weight and length reduction dependent on MV concentration	11, 20, 38, 1072, Marocco 239
IV	Seedlings weight and length reduction independent of MV concentration	1, 3, 4, 5, 6, 7, 12, 13, 14, 15, 17, 18, 19, 21, 23, 25, 27, 28, 29, 31, 32, 33, 35, 36, 37, 43, 44, 45, 46, 49, 159, 166, 169, 1005, 1009, 1011, 1012, 1013, 1019, 1020, 1023, 1025, 1028, 1029, 1030, 1031, 1034, 1038, 1039, 1044, 1045, 1047, 1050, 1062, 1067, 1069, 1070, 1073, 1076, 1077, 1082, 1084, 1085, 1088, 1089, 1092, 1093, 1094, 1095, 1096, 1100, 1102, 1104, 1105, 1107, 1111, 1112, 1113, 1115, 1118, 1119, 1121, 1122, 1123, 1124, 1127, 1128
V	Seedling length reduction independent of MV concentration, with no effect on seedling weight	Mauriso Fino, 2, 9, 1001, 1017, 1024, 1026, 1032, 1054, 1063, 1071
VI	Seedling weight reduction independent of MV concentration, with no effect on their length	1008, 1041, 1064

Subsequent type of response included 2 genotypes resistant to lower paraquat concentration and sensitive only to its higher concentration. In these forms, no inhibition of weight or length gain was observed as a result of treatment with lower MV concentration – seedlings grew well under these conditions. The negative effect of oxidative stress on the growth of seedlings was revealed only after using 10 μM MV.

Another type of response was characterized by a reduction in seedling weight and length depending on the MV concentration applied, i.e. negative effect of paraquat on seedling growth was observed at both applied stress factor concentrations, with a higher concentration of methyl viologen resulting in greater inhibition of plant growth.

The dominant type of response turned out to be the reduction of seedling weight and length independent of the MV concentration used (Fig. 2). In this case, inhibition of seedling growth was already observed under growth conditions in the presence of 5 μM MV, while the use of higher paraquat concentration did not further reduce the weight and length of seedlings; 58.8% of the tested forms showed this type of response.

The types of responses listed above belong to the overall plant response – they relate to the effect of oxidative stress on both weight and length of seedlings. As a result of the analysis of the obtained data, it was also possible to identify genotypes, in which the presence of paraquat reduced only one parameter, while no significant changes in the second parameter were observed.

Fig. 1. Genotype showing I type of response to oxidative stress. From left: 0, 5 and 10 μM methyl viologen treated seedlings

No significant effect of paraquat on the weight of seedlings, while inhibiting their growth in length was noted in 11 forms. It should be noted that higher MV concentration did not cause further inhibition of seedling growth. The opposite situation was observed in 3 other genotypes, where regardless of the MV concentration used, a decrease in seedling weight was observed, with no effect on their length. Thirty-one of the tested forms were characterized by a non-specific response to oxidative stress caused by MV application. The percentage share of genotypes in the specified types of responses to oxidative stress is presented in Figure 3.

Durum wheat is sensitive to stress factors. The tolerance of durum wheat genotypes to high temperatures was studied for instance by Bento et al. [2017]. Changing climate and an increase in average temperature will cause plants to be exposed to thermal stress [Semenov and Shewry 2011].

Of the 48 forms of Polish origin that were tested, only one (form No. 30: LGR 900/28/B) was insensitive to paraquat – seedlings of this form exhibited the same growth

under conditions of oxidative stress as under control conditions. In other forms originating from Poland, the presence of paraquat inhibited the growth in length and/or weight gain of seedlings. Seven forms were characterized by a non-specific response to MV-induced stress. Of the 40 sensitive forms, for which the type of response was determined, only one showed a response dependent on the concentration of the stress factor (type III response – form No. 11: No 1/269/82). As many as 32 genotypes originating from Poland responded by reducing the increase in length and mass as a result of exposure to lower MV concentration (type IV response). Six forms were classified into type V response (only length inhibition was observed, with no influence on seedlings weight), and one form into type VI response (inhibition of weight gain, with no effect on seedling length). Durum wheat genotypes are diverse in resistance and response to biotic and abiotic stress. Studies by Segit and Kociuba [2014] showed quite large diversity in the degree of durum wheat infestation by brown rust and leaf septoriosis. Over 50% of the forms with no symptoms of infection with these diseases were found. Low resistance to *Septoria* infection was observed. Of the 251 lines, 20 were qualified as useful in resistance breeding. Durum wheat sensitivity to low temperatures is also important. There was a great diversity in this trait in the *T. durum* genotypes studied by Diaz et al. [2019]. In the study, eleven genotypes were identified, which did not show any significant decrease in seedling weight and length under the influence of a stressor out of 148 forms.

Fig. 2. Genotype showing IV type of response to oxidative stress. From left: 0, 5 and 10 μM methyl viologen treated seedlings

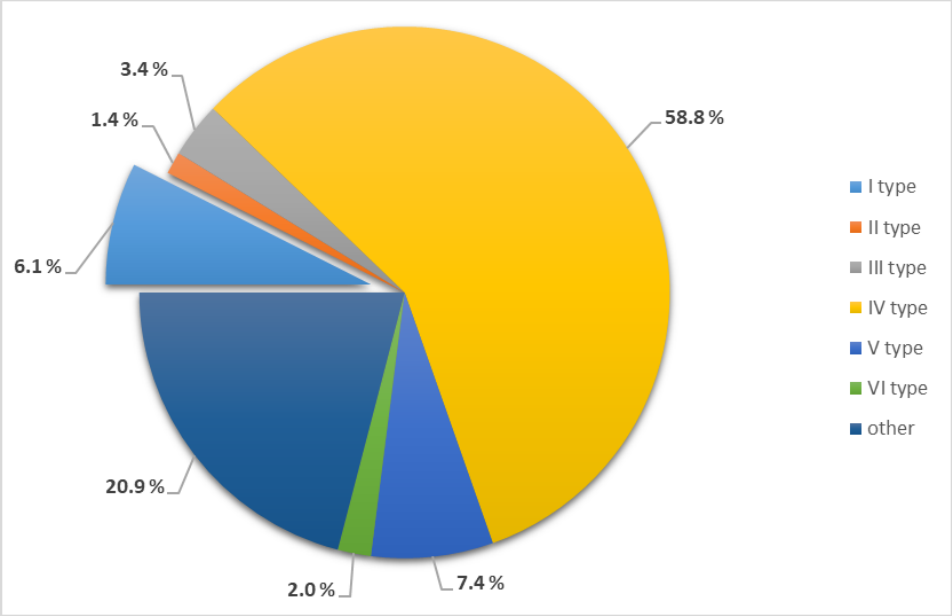

Fig. 3. Percentage share of studied genotypes in the specified types of responses to oxidative stress caused by the presence of MV

Damage to cells, tissues or organs caused by various stress factors can cause inhibition of plant development or ultimately lead to its death. The ability to survive under stress depends on the organism's resistance. In response to stress factors, plants have developed various defense mechanisms that allow the removal of reactive oxygen species and thus prevent or minimize cell damage. Therefore both the organism's adaptability, involving the activation and/or modification of mechanisms that support life functions under stress, as well as the organism's ability to repair damage caused by stress factor, are important [Małecka and Tomaszewska 2005, Mantri et al. 2012].

Of the tested cultivars and breeding lines of durum wheat, forms that have the highest and lowest resistance to oxidative stress are especially significant. This material can be used for further research aimed at identifying the mechanisms present in durum wheat, increasing its resistance to various types of abiotic and biotic stress. This is a valuable research perspective from the point of view of durum wheat breeding.

Many stress-inducible genes have been identified so far in Poaceae species. Among others, genes encoding for antioxidative enzymes are of particular interest. Study performed by Feki et al. [2016] showed induction of *TdMnSOD* gene in durum wheat plants subjected to osmotic, drought and H_2O_2-treatement. Moreover, the overexpression of the *TdMnSOD* gene in *Arabidopsis* resulted in its enhanced tolerance to multiple abiotic stresses. Another study by Feki et al. [2015] reported novel catalase gene from durum wheat – *TdCAT1*. Its expression in *Arabidopsis* also conferred tolerance to several abiotic stresses, thus confirming that *Triticum durum* might be a valuable source of resistance to multiple stresses.

CONCLUSIONS

The obtained results allowed the identification of forms resistant to oxidative stress among the tested cultivars and breeding lines of durum wheat. Selected genotypes can be used in resistance breeding aimed at obtaining cultivars grown for economy purposes characterized by increased resistance to stress factors that cause oxidative stress. In addition, the identified genotypes characterized by a diverse response to oxidative stress are valuable material from the point of view of future research aimed at identifying molecular mechanisms that increase resistance of durum wheat to various stress factors.

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Streszczenie. Stres oksydacyjny może powodować osłabienie lub obumarcie roślin. Mogą go wywoływać różne czynniki biotyczne i abiotyczne. W pracy analizowano genotypy pszenicy twardej w celu identyfikacji form charakteryzujących się podwyższoną tolerancyjnością na stres indukowany wiologenem metylu (parakwat). Obecność parakwatu w pożywce powodowała u większości analizowanych form zmniejszenie masy oraz długości części pędowej siewki, a także zahamowanie rozwoju systemu korzeniowego w porównaniu z formami kontrolnymi. Ponadto u niektórych z badanych form stres oksydacyjny powodował wystąpienie chloroz. Stwierdzono 6 różnych typów reakcji. Większość genotypów (58,8%) wykazywało obniżenie masy oraz długości siewek niezależnie od zastosowanego stężenia parakwatu. Zidentyfikowano 9 genotypów odpornych na działanie stresora (CYP, MEX × 2, ETH, FRA, ITA, POL, SUN, TUN), co stanowiło 6,1% badanych form.

Słowa kluczowe: pszenica twarda, stres oksydacyjny, wiologen metylu, parakwat

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APPENDIX A

Table A1. List of tested durum wheat (*Triticum durum* Desf.) forms

No.	Designation of the form in the collection	Genotype	Country of origin
1	*2*	*3*	*4*
1	1039	777	Afghanistan
2	1043	1774	Afghanistan
3	1044	1765	Afghanistan
4	1038	Shanazi	Afghanistan
5	1040	SAFAEDCHA	Afghanistan
6	26	GLORIE DE MONTGOLFIER	Algeria
7	1085	WINGS	Austria
8	18	SADOVO 5	Bulgaria
9	17	SADOVO 03	Bulgaria
10	31	CANDEAL 18	Chile
11	38	*T. durum*	Cyprus
12	37	*T. durum*	Cyprus
13	40	*T. durum*	Cyprus
14	1064	*T. durum*	Cyprus
15	1055	*T. durum*	Cyprus
16	1065	*T. durum*	Cyprus
17	1068	*T. durum*	Cyprus
18	1113	*T. durum*	Cyprus
19	1066	*T. durum*	Cyprus
20	1077	*T. durum*	Cyprus
21	1112	*T. durum*	Cyprus
22	1095	*T. durum*	Cyprus
23	1128	*T. durum*	Cyprus
24	1067	*T. durum*	Cyprus
25	1069	*T. durum*	Cyprus
26	1114	*T. durum*	Cyprus
27	1056	*T. durum*	Cyprus
28	1104	*T. durum*	Cyprus
29	1096	*T. durum*	Cyprus
30	1093	*T. durum*	Egypt
31	1041	*T. durum*	Egypt
32	41	*T. durum*	Ethiopia
33	42	*T. durum*	Ethiopia

1	*2*	*3*	*4*
34	12	*T. durum*	Ethiopia
35	46	Chandur	France
36	29	EXODUR	France
37	1105	Arstar	France
38	162	Aramon	France
39	50	Digital	France
40	4	PSATHAS	Greece
41	1076	GRIECHISCHER	Greece
42	1110	ISOPOURA	Greece
43	1111	EDITH	Israel
44	1	DURUM I	Jordan
45	3	D.T. 152	Canada
46	1115	MARROCOS No. 182	Morocco
47	6	MAROKKO 216	Morocco
48	7	MAROKKO 451	Morocco
49	8	MAROKKO 609	Morocco
50	9	MAROKKO 624	Morocco
51	1080	MAROKKO 534	Morocco
52	1123	BLEDURE 3225	Morocco
53	20	ROD 4610	Mexico
54	48	Rascon	Mexico
55	47	Ship	Mexico
56	45	Totanus	Mexico
57	13	ROD 4523	Mexico
58	1063	ROD 4573	Mexico
59	1124	ROD 4524	Mexico
60	1101	Gavza	Mexico
61	1127	ROD 4560	Mexico
62	1089	MEXICALI 75	Mexico
63	1087	Suraka	Mexico
64	1126	ROD 4520	Mexico
65	1092	CHEN „S”/18	Mexico
66	1079	PIMQUINO „S”	Mexico
67	159	Wegatail	Mexico
68	49	Sara	Mexico
69	14	ROD 4589	Mexico
70	15	ROD 4620	Mexico
71	1125	ROO 4623	Mexico
72	1119	ROD 4563	Mexico
73	36	LGR 900/51	Poland
74	35	LGR 900/48	Poland

Table A1 cont.

1	*2*	*3*	*4*
75	43	WGR 896/59B	Poland
76	28	LGR 1360/30a	Poland
77	25	K-5/876/84	Poland
78	32	LGR 898/43	Poland
79	33	LGR 899/7	Poland
80	30	LGR 900/28/B	Poland
81	1070	LGR 899/40	Poland
82	1084	LGR 898/4	Poland
83	1029	LGR 578/99/4	Poland
84	1031	LGR 579/99/6	Poland
85	1082	LGR 896/82/A	Poland
86	1073	No. 13/273/92	Poland
87	1032	LGR 576/99/5	Poland
88	1034	LGR 1359/8	Poland
89	1030	LGR 579/99/6	Poland
90	1122	K-12/790/84	Poland
91	1121	K-2/784/84	Poland
92	1023	LGR 520/99/4	Poland
93	1045	LGR 282/12	Poland
94	1046	LGR 282/12	Poland
95	1050	LGR 201/12	Poland
96	1025	LGR 635/99/10	Poland
97	1024	LGR 626b/99/1	Poland
98	1028	LGR 2426/98/2	Poland
99	1020	LGR 280/2	Poland
100	22	K-2/782/84	Poland
101	23	K-7/765/84	Poland
102	34	LGR 899/50	Poland
103	11	No 1/269/82	Poland
104	39	LGR 898/59	Poland
105	1026	LGR 635/99/7b	Poland
106	1054	LGR 317a/12	Poland
107	1009	LGR270/12	Poland
108	1017	246/12	Poland
109	1008	291/12	Poland
110	1001	305/12	Poland
111	1005	259/12	Poland
112	1011	220/12	Poland
113	1010	285/12	Poland

1	*2*	*3*	*4*
114	1012	311/12	Poland
115	1019	329/12	Poland
116	1013	286/12	Poland
117	1027	WGR 629/99/2	Poland
118	1094	LGR 900/17	Poland
119	1047	LGR 278/12	Poland
120	1022	LGR 900/3a	Poland
121	1078	2874	Portugal
122	21	AKMOLINSKA 2	Former USSR
123	1107	MEXICAN 01070	Former USSR
124	1062	MOURANICUM	Former USSR
125	1108	SZOP.TADINSKAJA 71	Former USSR
126	10	SANS	Tunisia
127	1118	KOKINI A	Tunisia
128	1057	AK-BASHAK 073-44	Turkey
129	1071	GEHUM	Turkey
130	24	G.K. BASA	Hungary
131	27	RNTAS	Italy
132	44	ALCHNTARA	Italy
133	1088	Valbelice	Italy
134	1100	Vespro	Italy
135	160	CAPELLI	Italy
136	1072	DIDI × THATCHER	unknown
137	169	Dura Negra	unknown
138	166	Florodur	unknown
139	Agridur	Agridur	unknown
140	Mauriso Fino	Mauriso Fino	unknown
141	Chirpan	Chirpan	unknown
142	Marocco 239	Marocco 239	unknown
143	ROD 4571	ROD 4571	unknown
144	19	UMMEDPUR WHEAT	unknown
145	5	RANCE	unknown
146	2	CROTONE	unknown
147	16	LOBEIRO 0342	unknown
148	1102	D 42	unknown

APPENDIX B

Significance of differences between group means of tested material (Tukey's HSD post-hoc test, $\alpha = 0.05$). Data marked with the same letter within the genotype is not significantly different.

Table B1. No response to oxidative stress, regardless of the MV concentration used

Genotype	MV concentration (μM)	Mean mass	Mean length
10	0	0,11[a]	13,58[a]
	5	0,07[a]	9,57[a]
	10	0,06[a]	9,60[a]
30	0	0,96[a]	12,79[a]
	5	0,93[a]	11,77[a]
	10	0,87[a]	11,69[a]
42	0	0,10[a]	14,67[a]
	5	0,10[a]	13,74[a]
	10	0,10[a]	14,10[a]
47	0	0,18[a]	14,33[a]
	5	0,12[a]	12,40[a]
	10	0,10[a]	10,50[a]
50	0	0,11[a]	14,00[a]
	5	0,08[a]	10,97[a]
	10	0,07[a]	9,98[a]
160	0	0,17[a]	18,90[a]
	5	0,07[a]	9,77[a]
	10	0,07[a]	16,6[a]
1108	0	0,10[a]	16,18[a]
	5	0,08[a]	15,76[a]
	10	0,07[a]	13,41[a]
1114	0	0,12[a]	16,70[a]
	5	0,07[a]	11,83[a]
	10	0,06[a]	10,43[a]
1125	0	0,10[a]	13,80[a]
	5	0,04[a]	8,73[a]
	10	0,02[a]	8,20[a]

Table B2. Reduction of weight and length of seedlings treated with higher MV concentration

Genotype	MV concentration (μM)	Mean mass	Mean length
1057	0	0,13[a]	16,78[a]
	5	0,07[a]	10,43[a]
	10	0,06[b]	9,44[b]
1101	0	0,19[a]	18,07[a]
	5	0,09[a]	12,28[a]
	10	0,17[b]	11,38[b]

Table B3. Seedlings weight and length reduction dependent on MV concentration

Genoptype	MV concentration (μM)	Mean mass	Mean length
11	0	0,17[a]	18,92[a]
	5	0,11[b]	14,58[b]
	10	0,08[c]	11,21[c]
20	0	0,21[a]	18,67[a]
	5	0,14[b]	15,11[b]
	10	0,10[c]	11,31[c]
38	0	0,17[a]	23,68[a]
	5	0,95[b]	16,13[b]
	10	0,06[c]	12,09[c]
1072	0	0,14[a]	17,02[a]
	5	0,09[b]	12,35[b]
	10	0,05[c]	8,08[c]
Marocco 239	0	0,25[a]	21,57[a]
	5	0,17[b]	17,68[b]
	10	0,10[c]	12,86[c]

Table B4. Seedlings weight and length reduction independent of MV concentration

Genotype	MV concentration (μM)	Mean mass	Mean length
1	*2*	*3*	*4*
1	0	0,14[a]	18,07[a]
	5	0,08[b]	11,33[b]
	10	0,07[b]	10,69[b]
3	0	0,16[a]	19,01[a]
	5	0,11[b]	13,37[b]
	10	0,10[b]	12,29[b]
4	0	0,16[a]	17,91[a]
	5	0,07[b]	10,42[b]
	10	0,07[b]	9,91[b]
5	0	0,12[a]	14,91[a]
	5	0,06[b]	9,44[b]
	10	0,05[b]	7,71[b]
6	0	0,16[a]	18,86[a]
	5	0,75[b]	11,10[b]
	10	0,07[b]	10,40[b]
7	0	0,23[a]	16,90[a]
	5	0,12[b]	13,02[b]
	10	0,10[b]	11,11[b]
12	0	0,15[a]	14,71[a]
	5	0,10[b]	9,50[b]
	10	0,10[b]	10,13[b]
13	0	0,17[a]	18,50[a]
	5	0,11[b]	13,40[b]
	10	0,09[b]	12,18[b]
14	0	0,18[a]	20,21[a]
	5	0,09[b]	13,01[b]
	10	0,09[b]	12,39[b]
15	0	0,17[a]	18,25[a]
	5	0,11[b]	13,27[b]
	10	0,10[b]	11,13[b]
17	0	0,22[a]	22,40[a]
	5	0,10[b]	12,63[b]
	10	0,06[b]	7,53[b]
18	0	0,17[a]	20,15[a]
	5	0,11[b]	14,43[b]
	10	0,09[b]	14,62[b]
19	0	0,21[a]	18,50[a]
	5	0,09[b]	10,60[b]
	10	0,06[b]	7,93[b]

1	*2*	*3*	*4*
21	0	0,16[a]	21,22[a]
	5	0,11[b]	18,10[b]
	10	0,10[b]	16,28[b]
23	0	0,20[a]	20,84[a]
	5	0,10[b]	12,86[b]
	10	0,09[b]	12,21[b]
25	0	0,16[a]	17,36[a]
	5	0,10[b]	12,73[b]
	10	0,09[b]	11,44[b]
27	0	0,13[a]	12,23[a]
	5	0,07[b]	9,09[b]
	10	0,05[b]	8,20[b]
28	0	0,15[a]	16,08[a]
	5	0,08[b]	11,20[b]
	10	0,06[b]	9,06[b]
29	0	0,18[a]	15,43[a]
	5	0,10[b]	11,94[b]
	10	0,10[b]	11,58[b]
31	0	0,17[a]	20,10[a]
	5	0,12[b]	14,29[b]
	10	0,10[b]	13,48[b]
32	0	0,18[a]	17,24[a]
	5	0,08[b]	10,83[b]
	10	0,07[b]	10,94[b]
33	0	0,17[a]	18,98[b]
	5	0,08[b]	11,64[b]
	10	0,07[b]	10,54[b]
35	0	0,18[a]	18,10[a]
	5	0,09[b]	11,93[b]
	10	0,08[b]	11,03[b]
36	0	0,14[a]	15,69[a]
	5	0,09[b]	10,47[b]
	10	0,08[b]	9,43[b]
37	0	0,11[a]	16,04[a]
	5	0,69[b]	11,36[b]
	10	0,63[b]	10,75[b]
43	0	0,13[a]	15,25[a]
	5	0,08[b]	11,17[b]
	10	0,06[b]	10,23[b]
44	0	0,18[a]	16,91[a]
	5	0,08[b]	9,72[b]
	10	0,08[b]	10,13[b]

Table B4 cont.

1	*2*	*3*	*4*
45	0	0,11[a]	12,95[a]
	5	0,07[b]	10,17[b]
	10	0,05[b]	8,25[b]
46	0	0,16[a]	14,14[a]
	5	0,09[b]	10,11[b]
	10	0,07[b]	8,54[b]
49	0	0,15[a]	15,84[a]
	5	0,10[b]	11,77[b]
	10	0,08[b]	11,08[b]
159	0	0,17[a]	16,39[a]
	5	0,08[b]	11,35[b]
	10	0,07[b]	10,37[b]
166	0	0,15[a]	17,19[a]
	5	0,09[b]	11,86[b]
	10	0,07[b]	10,07[b]
1005	0	0,16[a]	17,19[a]
	5	0,08[b]	11,43[b]
	10	0,08[b]	10,94[b]
1009	0	0,12[a]	15,69[a]
	5	0,06[b]	10,36[b]
	10	0,05[b]	9,93[b]
1011	0	0,15[a]	16,09[a]
	5	0,07[b]	10,05[b]
	10	0,07[b]	10,76[b]
1112	0	0,17[a]	17,96[a]
	5	0,08[b]	13,48[b]
	10	0,06[b]	10,13[b]
1013	0	0,16[a]	15,98[a]
	5	0,08[b]	10,79[b]
	10	0,07[b]	11,10[b]
1019	0	0,20[a]	19,78[a]
	5	0,11[b]	14,71[b]
	10	0,10[b]	12,89[b]
1020	0	0,17[a]	18,72[a]
	5	0,08[b]	14,61[b]
	10	0,07[b]	11,83[b]
1023	0	0,17[a]	15,62[a]
	5	0,07[b]	10,09[b]
	10	0,06[b]	8,41[b]
1025	0	0,19[a]	21,38[a]
	5	0,11[b]	14,74[b]
	10	0,09[b]	13,10[b]

1	*2*	*3*	*4*
1028	0	0,17[a]	16,60[a]
	5	0,11[b]	12,07[b]
	10	0,11[b]	12,04[b]
1029	0	0,17[a]	17,76[a]
	5	0,07[b]	10,35[b]
	10	0,07[b]	9,04[b]
1030	0	0,18[a]	20,54[a]
	5	0,09[b]	14,40[b]
	10	0,09[b]	12,77[b]
1031	0	0,15[a]	19,76[a]
	5	0,06[b]	10,68[b]
	10	0,05[b]	9,90[b]
1034	0	0,17[a]	18,02[a]
	5	0,06[b]	9,56[b]
	10	0,06[b]	9,71[b]
1038	0	0,17[a]	19,47[a]
	5	0,07[b]	9,68[b]
	10	0,05[b]	8,48[b]
1039	0	0,12[a]	18,36[a]
	5	0,07[b]	11,61[b]
	10	0,05[b]	11,19[b]
1044	0	0,22[a]	24,40[a]
	5	0,07[b]	12,91[b]
	10	0,06[b]	10,84[b]
1045	0	0,17[a]	16,92[a]
	5	0,06[b]	8,06[b]
	10	0,07[b]	9,97[b]
1047	0	0,19[a]	21,25[a]
	5	0,10[b]	12,10[b]
	10	0,08[b]	11,18[b]
1050	0	0,15[a]	17,32[a]
	5	0,05[b]	8,62[b]
	10	0,04[b]	7,85[b]
1062	0	0,22[a]	18,46[a]
	5	0,07[b]	10,78[b]
	10	0,07[b]	9,40[b]
1067	0	0,14[a]	18,24[a]
	5	0,04[b]	8,04[b]
	10	7,21[b]	8,04[b]
1069	0	0,17[a]	20,24[a]
	5	0,07[b]	12,96[b]
	10	0,07[b]	10,97[b]
1070	0	0,17[a]	17,13[a]
	5	0,07[b]	12,25[b]
	10	0,05[b]	8,30[b]

Table B4 cont.

1	*2*	*3*	*4*
1073	0	0,22[a]	23,40[a]
	5	0,08[b]	10,80[b]
	10	0,08[b]	12,20[b]
1076	0	0,18[a]	19,93[a]
	5	0,08[b]	11,62[b]
	10	0,07[b]	10,64[b]
1077	0	0,01[a]	17,68[a]
	5	0,04[b]	9,63[b]
	10	0,08[b]	7,74[b]
1082	0	0,21[a]	22,15[a]
	5	0,08[b]	13,00[b]
	10	0,08[b]	13,60[b]
1084	0	0,18[a]	15,67[a]
	5	0,06[b]	7,67[b]
	10	0,06[b]	7,84[b]
1085	0	0,15[a]	14,5[a]
	5	0,08[b]	9,87[b]
	10	0,06[b]	8,42[b]
1088	0	0,17[a]	18,41[a]
	5	0,11[b]	11,05[b]
	10	0,08[b]	9,42[b]
1089	0	0,16[a]	14,77[a]
	5	0,10[b]	11,83[b]
	10	0,09[b]	10,72[b]
1092	0	0,16[a]	14,99[a]
	5	0,07[b]	7,84[b]
	10	0,06[b]	7,43[b]
1093	0	0,18[a]	18,36[a]
	5	0,10[b]	13,61[b]
	10	0,08[b]	11,37[b]
1094	0	0,15[a]	14,89[a]
	5	0,08[b]	10,10[b]
	10	0,09[b]	11,50[b]
1095	0	0,13[a]	17,03[a]
	5	0,05[b]	9,42[b]
	10	0,07[b]	9,84[b]
1096	0	0,17[a]	20,24[a]
	5	0,07[b]	12,96[b]
	10	0,07[b]	10,97[b]
1100	0	0,14[a]	12,88[a]
	5	0,06[b]	8,51[b]
	10	0,52[b]	7,29[b]

1	*2*	*3*	*4*
1102	0	0,16[a]	18,89[a]
	5	0,11[b]	15,30[b]
	10	0,09[b]	13,46[b]
1104	0	0,15[a]	15,47[a]
	5	0,07[b]	9,33[b]
	10	0,06[b]	8,44[b]
1105	0	0,15[a]	14,95[a]
	5	0,09[b]	9,78[b]
	10	0,07[b]	8,23[b]
1107	0	0,13[a]	15,48[a]
	5	0,06[b]	8,31[b]
	10	0,08[b]	8,81[b]
1111	0	0,14[a]	16,72[a]
	5	0,05[b]	7,98[b]
	10	0,04[b]	7,27[b]
1112	5	0,14[a]	18,04[a]
	5	0,06[b]	10,9[b]
	10	0,06[b]	9,18[b]
1113	0	0,14[a]	18,57[a]
	5	0,06[b]	8,66[b]
	10	0,05[b]	9,29[b]
1115	0	0,13[a]	13,95[a]
	5	0,07[b]	9,33[b]
	10	0,06[b]	8,29[b]
1118	0	0,19[a]	18,77[a]
	5	0,05[b]	8,17[b]
	10	0,06[b]	8,93[b]
1119	0	0,18[a]	21,30[a]
	5	0,06[b]	10,00[b]
	10	0,60[b]	9,46[b]
1121	0	0,14[a]	17,68[a]
	5	0,07[b]	13,04[b]
	10	0,07[b]	12,22[b]
1122	0	0,17[a]	17,96[a]
	5	0,08[b]	13,48[b]
	10	0,06[b]	10,13[b]
1123	0	0,05[a]	7,96[a]
	5	0,04[b]	9,15[b]
	10	0,07[b]	10,30[b]
1124	0	0,18[a]	17,30[a]
	5	0,09[b]	9,31[b]
	10	0,08[b]	9,77[b]
1127	0	0,19[a]	19,54[a]
	5	0,11[b]	13,84[b]
	10	0,01[b]	13,69[b]
1128	0	0,22[a]	19,01[a]
	5	0,07[b]	11,91[b]
	10	0,06[b]	9,54[b]

Table B5. Seedling length reduction independent of MV concentration, with no effect on seedling weight

Genotype	MV concentration (μM)	Mean mass	Mean length
Maurisio Fino	0	0,14[a]	20,17[a]
	5	0,10[a]	16,28[b]
	10	0,12[a]	14,32[b]
2	0	0,13[a]	16,93[a]
	5	0,12[a]	11,83[b]
	10	0,09[a]	11,07[b]
9	0	0,20[a]	19,84[a]
	5	0,17[a]	12,94[b]
	10	0,08[a]	11,02[b]
1001	0	0,19[a]	18,10[a]
	5	0,09[a]	12,28[b]
	10	0,17[a]	11,38[b]
1017	0	0,15[a]	18,29[a]
	5	0,11[a]	10,56[b]
	10	0,06[a]	12,43[b]
1024	0	0,15[a]	16,79[a]
	5	0,11[a]	13,47[b]
	10	0,11[a]	11,97[b]
1026	0	0,13[a]	13,13[a]
	5	0,81[a]	10,63[b]
	10	0,08[a]	7,44[b]
1032	0	0,16[a]	16,58[a]
	5	0,15[a]	8,96[b]
	10	0,06[a]	9,83[b]
1054	0	0,15[a]	13,69[a]
	5	0,09[a]	9,91[b]
	10	0,08[a]	7,20[b]
1063	0	0,20[a]	18,80[a]
	5	0,17[a]	10,73[b]
	10	0,07[a]	10,54[b]
1071	0	0,15[a]	18,58[a]
	5	0,20[a]	12,34[b]
	10	0,06[a]	9,88[b]

Table B6. Seedling weight reduction independent of MV concentration, with no effect on their length

Genotype	MV Concentration (μM)	Mean mass	Mean length
1008	0	0,14[a]	14,71[a]
	5	0,08[b]	12,24[a]
	10	0,07[b]	11,08[a]
1041	0	0,16[a]	17,77[a]
	5	0,08[b]	12,10[a]
	10	0,07[b]	12,00[a]
1064	0	0,12[a]	17,35[a]
	5	0,07[b]	13,05[a]
	10	0,07[b]	13,15[a]